

## **The Metabolic Cost of Click Production in Bottlenose Dolphins**

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### **LONG-TERM GOALS**

Animals often increase the amplitude (the Lombard effect), duration, and/or repetition rate of their acoustic signals as a strategy to help reduce the probability of masking from environmental sounds (NRC 2003). Although accumulating evidence from recent research (Scheifele et al. 2005, Holt et al. 2009, Parks et al. 2010) illustrates that several marine mammal species readily modify the parameters of their acoustic signals to compensate for masking noise, potential energetic costs of such compensation behavior are unknown. To date, the only empirical data on the metabolic cost of sound production as well as the metabolic cost of increasing the amplitude of acoustic signals for any marine mammal species has been collected by the PIs during previously ONR-supported studies. The focus of the previous work was on whistle and social sound production in bottlenose dolphins (Holt *et al.* 2011 a, b, Noren *et al.* 2011). There is currently no information on energy expenditure during click production in odontocetes, and studies have demonstrated that they also readily modify these sound types in an echolocation context to compensate for masking noise. Given that changes in vocal behavior in response to masking noise has been documented in several species, assessing the biological significance of these effects is paramount but also very difficult given the life histories of marine mammals. The Population Consequences of Acoustic Disturbance (PCAD) model has been proposed as a framework to address this challenging task (NRC 2005). Data on the energetic cost of the production of clicks from this study can be used to assess the biological significance of vocal compensation in response to sound exposure and populate transfer function 2 (transfer function between behavior change to life functions immediately affected) in the PCAD model.

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## OBJECTIVES

The objective of this study is to measure oxygen consumption in two captive bottlenose dolphins to determine the metabolic cost of click production. The metabolic cost of click production will then be compared to resting metabolic rates, the metabolic cost of whistles and other communicative sounds, and the metabolic costs of other activities, such as performing surface active behaviors (SABs) and/or swimming. This work required two years to complete. Year 1 (training dolphins to perform the necessary behaviors and measuring metabolic rates during click production and resting trials) was initiated in 2012. For the second year of this study (ending in December 2013), we aimed to increase the number of experimental trials in order to quantify the metabolic cost of click production in bottlenose dolphins.

## APPROACH

The metabolic cost of click production is being measured in two captive male Atlantic bottlenose dolphins (*Tursiops truncatus*) maintained at Dr. Terrie Williams' Mammalian Physiology Laboratory at the University of California, Santa Cruz, Long Marine Laboratory. These individuals were trained by Traci Kendall (Program Manager/Research Training Supervisor) and Beau Richter (Head Trainer) to produce clicks on command while stationed under a metabolic hood to measure oxygen consumption. The sounds of free-ranging Atlantic bottlenose dolphins have been described as clicks, whistles, buzzes, quacks, and pops (Jacobs et al. 1993). The trained sounds of the captive dolphins of the current study are representative of those found in wild, free-ranging populations.

Experimental trials were conducted in the morning after an overnight fast to eliminate the potential for the metabolic cost of digestion to confound oxygen consumption measurements. Thus, food was given after the dolphin completed the entire experimental trial and only one experimental trial was conducted per dolphin per day. Dissimilar to the previous study to determine the metabolic cost of communicative sound (Noren et al. 2011, 2013), the dolphins produced clicks more consistently while under water (which is more typical of echolocating individuals in their natural environment). Thus, data were collected while the dolphins were slightly submerged during click production (they remained at the water surface during baseline, the 15 sec break between click bouts, and recovery). Briefly, each experimental trial consisted of three consecutive periods in which one dolphin entered the metabolic hood (details described in next paragraph) for (1) a 10-minute period of rest when the dolphin remained still and quiet at the water surface (to determine baseline metabolic rate), followed by (2) a click period performed slightly below the water surface but with otherwise minimal body movement and consisting of two consecutive one-min bouts of clicks separated by 15 sec of silence, and concluded with (3) a recovery period when the dolphin again remained still and quiet at the water surface (for at least 10 minutes, or until oxygen consumption values appeared to return to resting values). Because of the modification of the experimental protocol relative to the approach used for measuring the cost of communicative sound production, resting trials were also run in a manner that mimicked the total trial duration and submergence pattern of click production (experimental) trials. The total duration of resting trials were 22 min 15 sec and consist of three parts: 1) 10 min resting at the water surface, 2) one min resting slightly submerged, 15 sec resting at the water surface, one min resting slightly submerged, and 3) 10 min resting at the water surface. The purpose of conducting resting trials in this manner served as a control for the metabolic cost of submergence.

During all experimental trials, the dolphins were acoustically monitored in real-time and their sounds were recorded for further analysis as described below. The total durations of the rest period, click

period, and recovery period were recorded for each experimental session. Respirations were also recorded during each of the three periods so that respiration rates can be calculated for the dolphins during rest, click production, and recovery. The dolphin's behavior during each trial was also video recorded to ensure that body movement was kept to a minimum during all trial periods (baseline rest, click period, recovery). As mentioned above, separate control trials were also conducted to measure oxygen consumption and respiration rates during rest in the absence of click production. See figure 1 for a photograph taken during an experimental trial.



***Figure 1. Photograph taken during an experimental trial showing the equipment set-up which includes the metabolic hood, the dolphin stationed under the metabolic hood, the acoustic recording equipment and operator, the dolphin trainer, and the assistant taking notes and recording respirations. During the trial dolphin clicks were recorded via a hydrophone attached by to the melon by suction cup (not shown), oxygen consumption was continuously logged by an O<sub>2</sub> analyzer attached to a computer, and all respirations were recorded during each of the three periods.***

With the exception of dolphins producing sounds underwater in the present study, the method used to determine metabolic rates from oxygen consumption values is similar to those used previously on bottlenose dolphins producing communicative sounds (Noren et al. 2011, 2013). For this study, the rate of oxygen consumption ( $\dot{V}O_2$ ) was determined for quiescent dolphins stationed at the water surface and for the same dolphins producing clicks near the water surface. Air was drawn into the hood at a flow rate of 300 L min<sup>-1</sup>. The flow rate was maintained such that the content of oxygen in the hood remained above 20%. Water and CO<sub>2</sub> from subsamples of excurrent air from the hood were absorbed using Drierite and Baralyme, respectively, prior to entering the oxygen analyzer. The percentage of oxygen in the sample line was monitored continuously (FMS field metabolic rate system, Sable Systems International) and recorded by a laptop computer every second during experimental and

control trials.  $\dot{V}O_2$  for resting and clicking dolphins were calculated from the percentage oxygen data by respirometry software (Expedata data acquisition and analysis software, Sable Systems International). Because there may be metabolic costs associated with the brief breath hold bouts, the relative cost of breath hold bouts (determined from the resting trials) will be subtracted from the metabolic cost of click production (determined from the click production experimental trials) in order to isolate the cost of click production. Dr. Dawn Noren in collaboration with Dr. Robin Dunkin (post-doc) are responsible for collecting and analyzing the respiration rate and oxygen consumption data.

All experimental trials were acoustically monitored in real-time and also recorded using calibrated equipment to quantify the sound pressure level (dB re: 1  $\mu$ Pa), duration (in sec), the repetition rate (clicks/min), the number of clicks, and the frequency and energy content of the clicks produced by each dolphin during the experiment. A contact hydrophone was placed on the dolphin's melon during trials to carefully quantify these click parameters. This method was used because the dolphin is stationed at or close to the water surface under the hood and small changes in dolphin position can affect how much sound energy is transmitted under water. Recordings from the contact hydrophone will allow comparisons between trials and experimental conditions. The contact hydrophone consisted of a Reson TC 4013 hydrophone that was molded into a small suction cup for contact. The contact hydrophone was then connected through a bandpass filter and amplified (Reson VP 2000), and the signal was sent through a DAQ device (IOtech Personal DAQ 3000) which digitized the signal at a sampling rate of 500 kHz. The sound files were stored on a PC laptop for further analysis. Hydrophone placement was the same for each dolphin and during all periods (rest, click production, and recovery) of each experimental session. All sounds produced during trials are analyzed using Avisoft SASlab Pro (v5.1.17) and/or Matlab (R2011b or higher versions). Dr. Marla Holt is responsible for collecting and analyzing the acoustic data.

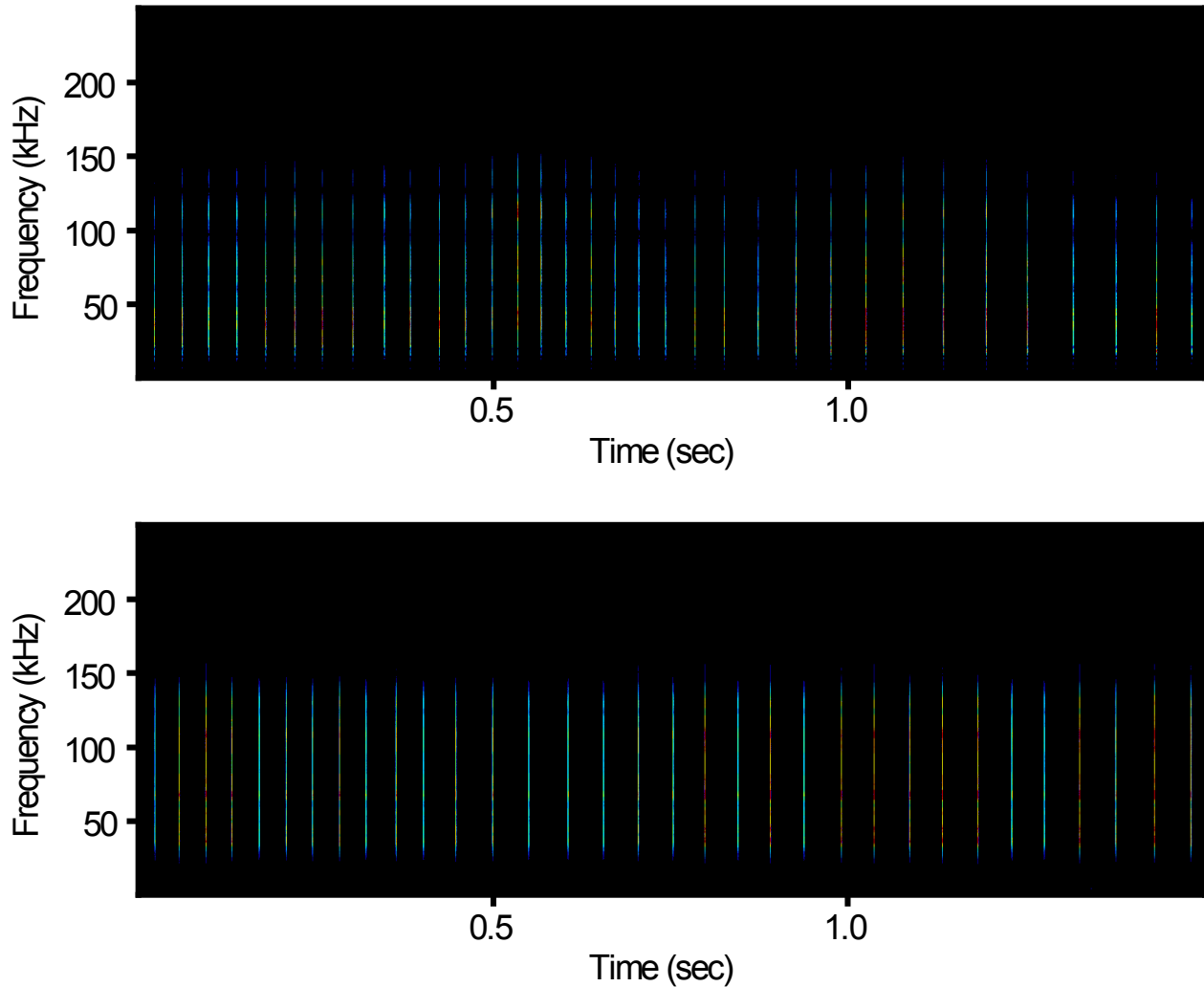
## **WORK COMPLETED**

Since our previous report (FY12 MBholt2) four full week trips to Dr. Terrie Williams' Mammalian Physiology Laboratory at the University of California, Santa Cruz, Long Marine Laboratory were conducted to complete data collection for the project. During these trips, which occurred in mid-October 2012, late January 2013, early February 2013, and late February 2013, we ran an experimental (click production) trial every day for a total of 20 experimental trials per dolphin. Control (resting) trials were also conducted 2-3 times per month between mid-October 2012 and early March 2013, resulting in 16 control trials per dolphin. Together with trials run for this project in FY12, we have run a total of 42 experimental trials per dolphin, although many of the ones run in FY12 will not be included in data analysis because of variable click performance or other experimental issues. In addition, preliminary analysis of the oxygen consumption data collected during experimental and resting trials have been conducted and the calculation of respiration rates are nearly complete.

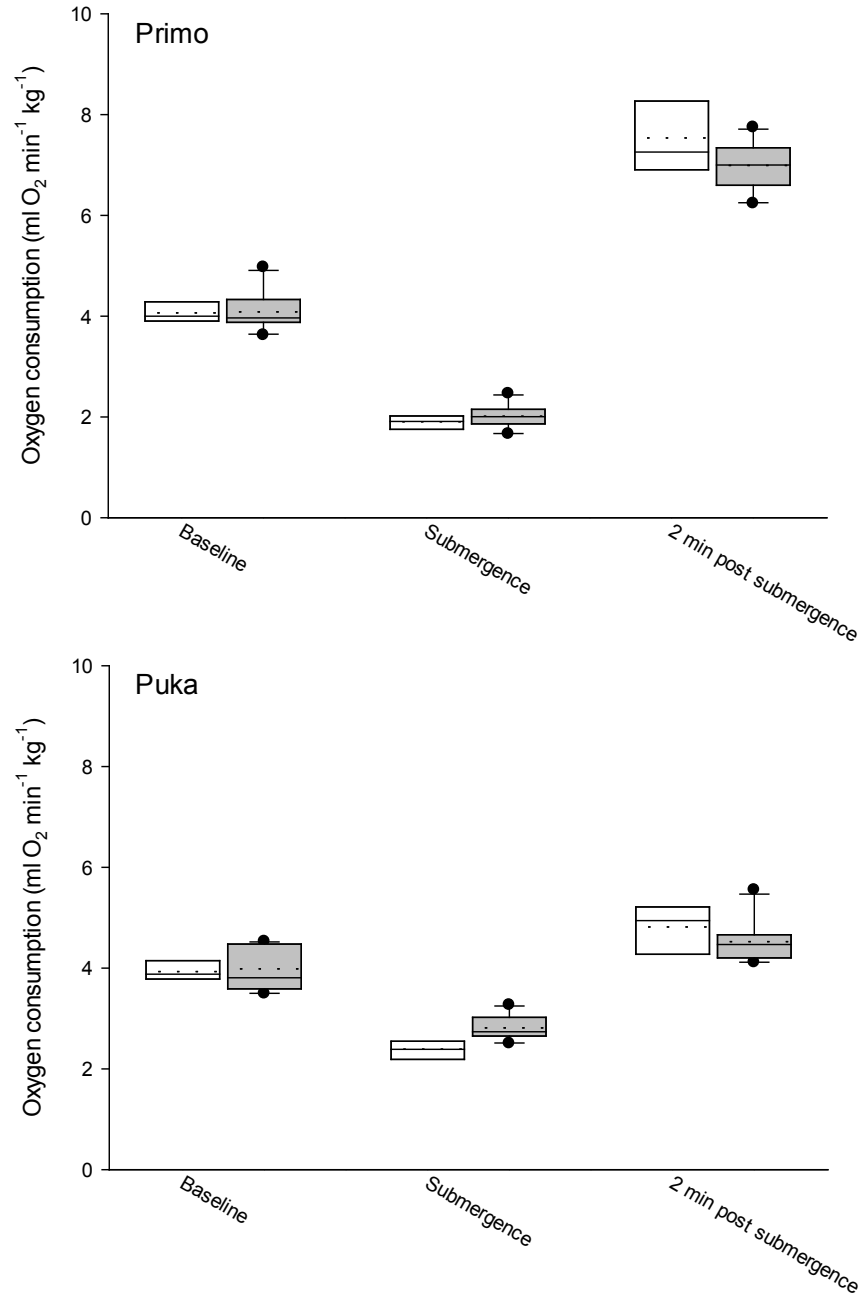
## **RESULTS**

Data collection for the project has now been completed. Forty experimental trials were conducted (20 per dolphin) since our last report (FY12 MBholt2) and data from almost all of these trials will be included in the analysis. Example spectrograms for dolphins producing clicks during experimental trials (Fig. 2) and preliminary results of oxygen consumption rates during three components of experimental and resting trials conducted from late January through early March 2013 are presented (Fig. 3). Preliminary results demonstrate that there may be a small oxygen debt accrued during

submergence from breath holding that is then compensated for upon surfacing in both resting and experimental trials. This cost will be removed from experimental trials to assess whether there is a significant metabolic cost of producing clicks. The preliminary results suggest that there may be a small, but measurable cost of click production for at least one of the dolphins, but statistical tests were not conducted on this small subset of data. Statistical analyses will be conducted on the full set of data once the quality of all control and experimental trials have been assessed.



***Figure 2. Spectrograms showing 1.5 second examples of clicks performed by Primo (top panel) and Puka (bottom panel) as recorded by a contact hydrophone placed on the melon of the dolphin. Both spectrograms show visual representations of clicks produced during oxygen consumption data collection with time from 0-1.5 seconds on the x-axis and frequency from 0-250 kHz on the y-axis. The colors denote relative level or amplitude differences with red indicating higher levels and blue indicating lower levels.***



**Figure 3.** Oxygen consumption (ml O<sub>2</sub> min<sup>-1</sup> kg<sup>-1</sup>) during three components of control and experimental (click production) trials for two dolphins in late January – early March 2013. Control (n=6) and experimental (n=10) trials are designated by white and gray bars, respectively. For each box plot, the boundary of the box closest to zero indicates the 25th percentile, the solid line within the box marks the median, the dashed line within the box marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively, and the dots designate outlying data points. Because this is only a subset of all of the data collected, no statistical tests were run on the data.



## IMPACT/APPLICATIONS

Currently, there is no empirical data on the metabolic cost of click production in any delphinid species. Theoretical assessments of such costs need to factor in variables such as efficiency factors and the relationships between physiological processes and metabolic costs associated with behaviors given that they often do not simply scale according to linear relationships. However, such data needed for theoretical modeling on this topic are also lacking. Empirical data collected from this study will provide valuable information about the cost of click production in odontocetes and will be useful in assessing potential costs of modifying click production in response to anthropogenic sound exposure. Specifically, this study will provide important input data to populate transfer function 2 in the PCAD model which can then be used to assess the biological significance of such responses to anthropogenic sound exposure.

## RELATED PROJECTS

Dr. Terrie Williams' Marine Mammal Physiology Project involves other studies on the two dolphins used in this study. The goal of one related study is to assess the changing energetic demands in cetaceans, and in particular, determine the principle factors in regulating the variable metabolism of cetaceans over the seasons.

[http://www.mmpp.ucsc.edu/The\\_Marine\\_Mammal\\_Physiology\\_Project/Home.html](http://www.mmpp.ucsc.edu/The_Marine_Mammal_Physiology_Project/Home.html)

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